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Memorandum

Date September 26, 1991

From Chief, Hematology/Pathology Branch, Division of Clinical
Laboratory Devices (HFZ-440), Office of Device Evaluation, Center
for Devices and Radiological Health

Subject Draft Guidance for 510(k) Submission of Lymphocyte
Immunophenotyping Monoclonal Antibodies

To Interested Manufacturers

We have developed a draft document entitled, "Guidance Document for 510(k) Submission of Lymphocyte Immunophenotyping Monoclonal Antibodies." Since the document lists items we will be reviewing, it is intended to assist manufacturers in the preparation of marketing submissions for these types of devices.

As this area of in vitro diagnostics is rapidly expanding in the clinical laboratory, we are soliciting your ideas, recommendations, and comments regarding the enclosed draft guidance document. We will appreciate receiving your comments so we can incorporate as many improvements as possible in a revision. Additional copies of this document may be obtained through the Office of Small Manufacturers Assistance from Geoffrey Clark, (301) 443-6597.

Please address comments to:

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DRAFT: Guidance Document For 510(k) Submission of Lymphocyte
Immunophenotyping Monoclonal Antibodies.

This is a flexible document representing the current major concerns and suggestions regarding lymphocyte immunophenotyping in vitro diagnostic devices employing monoclonal antibodies methodology with flow cytometry, fluorescence and light microscopy. It is based on 1) current basic science, 2) clinical experience and 3) previous submissions by manufacturers to the FDA. As advances are made in science and medicine, these review criteria will be re-evaluated and revised as necessary to accommodate new knowledge.

REVIEW CRITERIA FOR ASSESSMENT OF LYMPHOCYTE IMMUNOPHENOTYPING IN
VITRO DIAGNOSTIC DEVICES USING MONOCLONAL ANTIBODIES WITH FLOW
CYTOMETRY, FLUORESCENCE AND LIGHT MICROSCOPY

For the purposes of this guidance document lymphocytes are defined as those lymphocytes possessing the cluster of differentiation (CD)2, CD3, CD4, CD5, CD8, CD16, CD19, CD20, and CD56. This document also covers CD45, CD14, and CD13 which are used for lymphocyte gating and HLA-DR used for the detection of activated CD3 positive cells. This document also covers anti-isotype controls, and secondary antibodies labeled with biotin or a chromogen (any compound which is not a dye but is capable of becoming one). In this context, fluorescein isothiocyanate (FITC), phycoerythrin (PE), other fluorochromes, and horseradish peroxidase, etc. are chromogens.

PRODUCT CODE: GKZ

REGULATION NUMBER: 21 CFR § 864.5220

PANEL: Hematology/Pathology

CLASS: II

REVIEW REQUIRED: 510(k)

DEFINITION: This generic type device is intended for use in clinical laboratories as an in vitro test for the quantitative measurement of lymphocytes and their subsets using monoclonal antibodies (Mab) by flow cytometry, light and fluorescence microscopy.

PURPOSE: The purpose of this document is to provide guidance on information to present to the Food and Drug Administration (FDA) before a device utilizing MAb's to immunophenotype lymphocytes may be cleared for marketing. This information enables FDA to make better informed decisions based on a uniform data base.

Where applicable this document references sections from the National Committee for Clinical Laboratory Standards (NCCLS) Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes.⁽¹⁾ If not otherwise defined, the definitions for various terms used in this document are taken from the NCCLS document.

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I. Background.

Current clinical use of the above MABs is for enumeration (percentage) of the CD either as a single subset or a group within a subset using multi-parameter and multi-color methodology. Immunophenotyping lymphocytes with MABs can be used as an aid in the determination of the immune status. Much work has been done with CD4 and CD8 values in their relation with HIV (human immunodeficiency virus) infection and the progression of acquired immune deficiency syndrome (AIDS) and other immune deficiency diseases. Enumerating total CD4 positive and CD8 positive lymphocytes in HIV infected individuals allows the physician to place these individuals into various staging groups.^(2,3) The clinical significance of lymphocyte immunophenotyping MABs has been shown for this situation. At present, the only intended use for which lymphocyte immunophenotyping MABs are cleared is the enumeration of lymphocyte subsets. The clinical significance of any subset associated with leukemias and lymphomas has yet to be universally accepted. A change of the intended use of the above referenced MABs from enumeration of normal lymphocyte subsets in human blood to a more specific intended use of typing various leukemias and lymphomas would require a premarket application for approval to market (PMA). This would be considered a new intended use for these devices and would require a more rigorous process for approval. The clinical significance for MABs other than the above uses has not been shown or has not been widely accepted. The PMA procedure may also include MABs for activation markers (other than CD3+/HLA-DR), platelets, other leukocytes, and precursor human blood cells.

Exceptions to the above are the MABs which recognize CD45, CD13, CD14 and keyhole limpet hemocyanin used for anti-isotype controls. Although, some clinical relevance may be placed on the variation of these CDs in a patient, their primary uses at this time are quality control (QC) and as an aid in gating of samples on a flow cytometer. An intended use for enumeration of lymphocytes for these MABs is not appropriate at this time.

II. Device Description.

Monoclonal antibodies are directed against specific CD epitopes on human lymphocytes. The MABs may be used alone or in conjunction with each other, labeled or unlabeled. The MABs are either labeled (having a chromogen or avidin attached) or unlabeled (to be used with a secondary antibody labeled with an appropriate chromogen). The use of a biotin-avidin complex and labeled secondary antibody is normally used for amplification of a weak signal. More than one MAB may be present to enumerate specific groups within a subset of lymphocytes, such as, CD8+/CD11b+, CD8+/CD11b-, CD8-/CD11b+, and CD8-/CD11b- cells. Additionally, three chromogens may be used to further enumerate subsets, such as CD8+/CD11+/CDx+.

A. Principle of the Test.

A specific MAB is added to a sample of whole blood or

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isolated mononuclear cells. After an appropriate incubation period and washes, the lymphocytes which have been labeled with the antibody/chromogen complex are enumerated. Depending upon the method used (cytometry or microscopy), the chromogen is detected with light excitation or chemical color change.

B. Specimen Type(s).

Anticoagulated whole blood; Lymphocytes are isolated from erythrocytes by accepted methods such as whole blood lysis or gradient centrifugation. Anticoagulants encompass all of the acceptable anticoagulants used for specimen collection of whole blood for lymphocyte immunophenotyping. NCCLS lists the following anticoagulants; ethylenediaminetetraacetic acid (EDTA), heparin, and acid citrate dextrose (ACD).⁽¹⁾ If a manufacturer wishes to make a claim for an anticoagulant other than the above, comparison data is needed for the new anticoagulant with the anticoagulants listed above. Lymphocytes are either fixed (labeled samples suspended in buffered formaldehyde, pH 7.2 or paraformaldehyde), or unfixed and contain azide.

III. Specific Performance Characteristics.

The FDA requires different types and amounts of data and statistical analyses to be included in a manufacturer's application for marketing of in vitro diagnostic devices. Additional data may be necessary to substantiate certain claims of intended use or clinical significance. If data presented in the submission adequately demonstrates there is no significant difference in test results between different sample types/matrices using appropriate statistical studies; e.g., regression studies or paired T tests, then test performance characteristics may be determined with one specimen of choice. Otherwise, performance characteristics must be demonstrated for all sample types/matrices claimed for use or which demonstrate statistical differences.

In addition to proof that the MAb clone being submitted has been classified by the World Health Organizations' International Workshops on Human Leucocyte Differentiation Antigens, the submission of the following data is required to determine substantial equivalence of MABs for immunophenotyping of lymphocytes using flow cytometry, fluorescence, or light microscopy:

A. Analytical/Laboratory/In Vitro Studies.

Specific parameters of importance to the use of the MAB should be supported by data determined with that MAB clone. It should be demonstrated that the performance of the MAB is substantially equivalent to another legally marketed device. Present test data with analyses and conclusions. The data and statistical evaluation should be sufficient to determine if the device is substantially equivalent and/or safe and effective for all claimed specimen type(s)/matrices.

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Address the following performance characteristics in a submission for lymphocyte immunophenotyping using flow cytometry or fluorescence/light microscopy:

1. Performance Characteristics.

- a) **Antibody Specificity (For Immunological Assays).**
Investigate the cross-reactivity and interference of any potentially interfering substances using the assay system. Submit data documenting any crossreactivity of the antibodies with the following cell populations:

- * monocytes
- * granulocytes
- * platelets
- * red blood cells

Additional data should be submitted to demonstrate that MABs in combination are equivalent to the single MABs alone.

- b) **Linear Range.**
Determine the linear range of the assay using 4 each low, normal, and abnormal specimens. Demonstrate the range by linear regression and 95% confidence intervals of the data points.
- c) **Accuracy/Recovery Studies.**
Determine the spiking recovery of known amounts of the target lymphocyte subset (CDx). This may be accomplished by doing limiting dilutions of lymphocytes containing the subset of interest.

2. Reproducibility Studies.

- a) **Intralaboratory Reproducibility.**
Determine the within-day precision using one flow cytometer, the new antibody and patient samples with three levels of CDx positive lymphocytes; high, normal, and low. Samples outside of the normal range can be obtained by diluting normal samples with known CDx negative cells. Run the samples in replicates of ten.
- b) **Interlaboratory Reproducibility.**
Repeat the intralaboratory study at three independent sites.

A summary of the results from the intra- and interlaboratory reproducibility studies should be reported in the Performance Characteristics Section of the Package Insert.

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B. Clinical Comparison Studies.

Comparison studies provide data on the ability of the system to accurately determine MAb immunophenotyping results as compared to another legally marketed device. For comparison of flow cytometry data, the clinical sites should furnish the protocol for instrument calibration, instrument setup for analysis, and isolation of cells of interest. Each site performing the following studies should use the NCCLS Standard, Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes.⁽¹⁾

1. Specimen Collection and Handling.

Collect patient blood samples following the venipuncture procedure outlined in the NCCLS Standard, Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture.⁽⁴⁾ For immunophenotyping, the anticoagulants EDTA, ACD solution A, or heparin may be used. Identify the blood specimen with a unique patient identifier, date, time of collection, sex, age, and pertinent medication information. Maintain blood samples at 18-22°C and process within 6 hours of collection. For flow cytometric analysis, samples may be fixed in a solution of 1% paraformaldehyde or 1% buffered formaldehyde, pH 7.2. State specimen storage conditions in the Package Insert and provide data or appropriate literature references in the submission to substantiate any claims made.

2. Expected Normal Ranges.

Analyze anticoagulated whole blood samples from a minimum of 50 normal donors from each of three geographically diverse sites which may include the manufacturer's own site. These donors should include all ethnic groups (White, Asian, Hispanic, and Black, both U.S. and Caribbean) as well as represent both genders and a broad defined age range. Test the blood samples with both the new MAb and the comparable legally marketed MAb.

Test each cell isolation protocol claimed in the Intended Use Statement with the 50 samples; e.g., Ficoll-hypaque mononuclear cell separation, buffy coat, and whole blood lysis.

Establish a normal range of CDx positive lymphocytes using the new test with samples from a minimum of 50 normal persons. Characterize the population study according to age, sex, geographic location, and symptoms of disease. State the statistical method used in the package insert. All sample type(s)/matrices should be considered in the studies.

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3. Abnormal Ranges.

Support any claims or references to disease states with data. Depending on the rarity of the disease, test a minimum of 10 (more would be preferred) samples according to the protocols submitted. The sampling for these disease states should be as diverse as possible. Continuous cell lines of the appropriate lineage may be used to enhance the data if abnormal samples are extremely hard to obtain. Determine the range of CDx positive lymphocytes from each specific claimed disease state.

4. Flow Cytometer.

If the intended use claim is not for a specific manufacturer's flow cytometer, a comparison must be done using both a Coulter and Becton-Dickinson instrument as these are considered to be the most commonly used instruments. For the comparison study use 20 to 30 patient samples which contain an evenly distributed number of high, normal, and low levels of cells. Samples outside of the normal range can be obtained by diluting normal samples with known CDx negative cells. The comparison samples should be the same for all instruments and may consist of continuous cell lines possessing the antigen of interest. Submit a representative number of fluorescence intensity histograms for all flow cytometers used.

C. Presentation of Data.

Present all data as percentage of the total lymphocyte population, listed in tabular form and sorted based on the submitted device.

Provide the linear regression for the comparison data with the predicate device and, also, for each machine comparison. List the mean channel fluorescence of the histograms for the generic flow cytometer comparison data.

Include the calculations from the normal and disease states for the mean, SD and the percent coefficient of variation (CV).

Show the data from the disease states as a total and listed by age, ethnic group, and sex.

Submit a representative number of fluorescence intensity histograms for all flow cytometers used.

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IV. Labeling Considerations.

A. Suggested Intended Use Statement.

A typical intended use statement is: "ABC's Bright Star CDx murine monoclonal antibody is for the identification and enumeration of CDx positive lymphocytes in whole blood using flow cytometry."

B. Clinical Utility.

The clinical utility for the MAb should be included in the Intended Use Statement.

C. Limitation.

The following limitation statement should be included in the Package Insert:

Due to an unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary.⁽⁵⁾

V. References.

1. National Committee for Clinical Laboratory Standards. Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes; proposed guideline. Villanova, PA. 1989;9(13). Order code H42-P.
2. Redfield RR, Wright DC, Tramont EC. The Walter Reed Staging Classification for HTLV-III / LAV Infection. N Engl J Med 1986;314(2):131-132.
3. CDC. Classification System for Human T-Lymphotropic Virus Type III / Lymphadenopathy - Associated Virus Infections. MMWR 1986;35(2):334-339.
4. National Committee for Clinical Laboratory Standards. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; approved standard. Villanova, PA. 1984;4(5). Order code H3-A2.
5. Koepke JA, Landay AL. Precision and Accuracy of Absolute Lymphocytes Counts. Clin Immunology and Immunopathology 1989;52:19-27.